The Candida in Pregnancy Study (CiPS)

ANZ Trial Registration ID: ACTRN12610000607077

Statistical Analysis Plan

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Data sharing – The investigators acknowledge, and concur with, the research community's wish to publicly share trial data. Outcome data for this trial are obtained from routinely collected data that are maintained and administered by the Ministry of Health, with access predicated by the Ministry rules and regulations. To protect patient privacy, the ethics approval and data release requirements for this trial prohibit the trial researchers reporting or sharing individual patient data with other researchers, only summary data can be published or shared. Summary (aggregated) data can be provided to other researchers, as long as such data cannot result in identification of any individual patient. Any group wishing to access anonymised individual patient record data for research purposes must seek approval from the relevant Human Research Ethics Committees and, subject to ethical approval, the NSW Ministry of Health (for data release).

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List of Abbreviations

Abbreviation	Definition
APDC	Admitted Patients Data Collection
CI	Confidence intervals
CiPS	Candida in Pregnancy Study
CONSORT	Consolidated Standards of Reporting Trials
DMC	Data Monitoring Committee
GA	Gestational age
GDM	Gestational diabetes mellitus
ICU	Intensive care unit
MOH	Ministry of Health (NSW)
NICU	Neonatal Intensive care unit
NSW	New South Wales
PDC	Perinatal Data Collection
PPROM	Preterm prelabour rupture of membranes
PROBE	Prospective, Randomised, Open-label, Blinded Endpoint
RR	Relative risk
SGA	Small for gestational age

1. BRIEF BACKGROUND

1.1 Trial overview

Prevention of preterm birth remains an important challenge in maternity care. Recognition that ascending infection leads to preterm birth has led to a number of studies that have evaluated the treatment of vaginal infections in pregnancy to reduce preterm birth rates. In studies utilising population-based data from Hungary treatment of vaginal candidiasis during pregnancy was associated with a 34% to 64% reduction in the prevalence of preterm birth.[1-3] In contrast, two cohort studies found no significant association between preterm birth and moderate to heavy growth of *Candida* species among women at 22 to 30 weeks gestation.[4,5] A recent systematic review found two trials comparing the treatment of asymptomatic vaginal candidiasis in pregnancy for the outcome of preterm birth.[6] Although the effect estimate (meta-analysis RR = 0.36, 95% CI = 0.17 to 0.75) suggested that treatment of asymptomatic candidiasis may reduce the risk of preterm birth, the result needs to be interpreted with caution as the primary driver for the pooled estimate comes from a post-hoc (unplanned) subgroup analysis of a broader infection screening trial.[7] A prospective trial with sufficient power to answer the clinical question 'compared to usual care, does treatment of asymptomatic candidiasis in early pregnancy prevent preterm birth' is warranted.[6]

Consequently we have undertaken a prospective, randomised, open-label, blinded endpoint (PROBE) trial[8,9] with a simple *Candida* testing protocol that can be easily incorporated into usual antenatal care; a simple, well accepted, treatment intervention; and assessment of outcomes from validated, routinely-collected, computerised databases. The trial is registered with the Australia and New Zealand Clinical Trial Register (ANZCTR) as ACTRN12610000607077. Collection of outcome data from existing data sources is a novel and cost-efficient approach for assessing outcomes that reduces the burden on participants and clinical research staff while maintaining data quality. We have previously demonstrated that the outcomes for this study are reliably collected in routinely collected obstetric and hospital databases[10-18] and that record linkage allows identification of maternal transfers to another hospital to determine outcomes for these few women who would otherwise be lost to follow-up.[17,19]

1.2 Estimated timeline for the trial

August 2010	Trial protocol submitted for publication in a peer-reviewed open access journal
December 2010	First participant recruited to the trial
March 2011	Trial protocol published in open access journal
January 2016	50% of study population has given birth
July 2016	Finalisation and publication (University of Sydney open access repository) of Statistical Analysis Plan
August 2016	Outcome data will be available for the interim analysis to be performed by the Trial Statistician under the direction of the Data Monitoring Committee with a decision on trial continuation
August 2017	Anticipated date of final participant recruitment
March 2018	Anticipated date final participant gives birth
December 2018	Anticipated dataset lock, unblinding of treatment assignment

1.3 Aim

To conduct a randomised controlled trial to answer the clinical question*: In women with asymptomatic vaginal candidiasis early in pregnancy does treatment with clotrimazole prevent spontaneous preterm birth <37 weeks gestation.*

Hypothesis: that treatment of asymptomatic vaginal candidiasis with clotrimazole is associated with a 40% reduction in spontaneous preterm birth <37 weeks gestation.

1.4 Study Design

This is a prospective, randomised, open-label, blinded endpoint (PROBE) study design conducted in 9 maternity hospitals in New South Wales, Australia. The protocol for the trial was published in an open access journal.[20] Eligible asymptomatic women are enrolled in the study and a vaginal swab is self-collected. If the swab culture is positive for *Candida* species (at least one colony formed on agar plating), women are randomised to either treatment or usual care. Randomisation is centrally administered by a researcher not involved in patient care. The 1:1 randomisation schedule was computer generated using permuted blocks, stratified by recruiting hospital. Women randomised to treatment are notified by phone or email (dependent on each participants preference, specified at trial entry), and a central pharmacy dispenses the study medication which is mailed to women in the treatment arm, usually within 5 days of the swab collection. An interim analysis will be conducted when birth

outcomes are potentially available for the first 50% of women recruited to the study, and a decision on study continuation made at this point.

1.5 Eligibility criteria

Pregnant women with a singleton pregnancy presenting for antenatal care prior to 20 weeks gestation are eligible for *Candida* screening and inclusion in the study. Women presenting beyond 20 weeks gestation or with a history of hypersensitivity to clotrimazole are excluded. Of note, some women may have their gestational age revised and/or a multiple pregnancy diagnosed after enrolment in the study, but will be retained in the study. Women with symptomatic vaginal infection due to *Candida* are ineligible for the study as they require treatment.

1.6 Intervention

Treatment group: Women randomised to treatment are notified of their screening result by a researcher not involved in patient care. A 6-day course of the study medication, commercially available vaginal clotrimazole pessaries (Canestan®) is then mailed to the women. The women are advised to insert one pessary as gently and deeply as possible into the vagina, preferably at night, for 6 nights. Women who miss one or more daily doses are advised to insert a single pessary as soon as they remember or the next evening, and continue using the pessaries until the course of treatment is finished.

Usual care: Women randomised to usual care are not notified of their screening result, and receive no treatment other than usual care.

Women in both treatment arms and their babies are managed according to standard practices of the model of maternity care that they have chosen. Women developing symptoms of *Candida* infection are offered treatment. Results of the initial screening swab are not reported to the health care provider nor recorded on the patient's pregnancy record.

1.7 Data collection and follow up

On enrolment into the study (referred to as screening), baseline trial data are collected by a research midwife using a standardised form (see Appendix 1). Additional baseline data are obtained from existing obstetric and hospital discharge databases. Data on the primary and secondary outcomes are also collected from these existing computerised databases. Women can deliver at hospitals other than the hospital they were recruited at, or as unplanned home births or have a miscarriage. Statewide record linkage is used to determine

outcomes for women who do not birth at a recruiting hospital. Because determining outcomes from routinely collected data is a novel approach, we planned *a priori*, to verify the primary outcome by reviewing the medical records of all women identified in the computerised databases as delivering preterm (details page 9).

A brief follow-up survey for additional secondary outcomes is completed as a web-based questionnaire with email notification, or mailed with a reply-paid envelope according to the participant's stated preference at trial entry. These separate study data are collected after 28 weeks of gestation, when the potential for unmasking the 'no treatment' arm to the participant is irrelevant.

The data sources used in the study are described in more detail below:

Trial data

CiPS trial data, collected during the initial screening process, include: date and gestational age at the time of screening, the results of candidiasis screening, date of randomisation (enrolment into the trial), treatment allocation (to remain concealed from analysis until the trial is completed) and follow-up survey data. Follow-up survey data include information about symptoms of candidiasis, use of antibiotics and/or corticosteroids, treatment of candidiasis (both arms of the trial), treatment side-effects, and compliance with the treatment regimen.

In addition, the trial coordinator keeps a log of *personal contacts* (phone and email) with participants. These contacts include notification that a participant had suffered a pregnancy loss or moved and birthed elsewhere (another hospital in NSW or outside the state). Every effort is made to determine gestational age at delivery for all women who were randomised in the study.

Obstetric databases are accessed at both a hospital and statewide level. At the hospital level data are extracted from each participating hospital's computerised system. These databases collect information about all livebirths or stillbirths ≥20 weeks' gestation or ≥400g birthweight including maternal characteristics, antenatal care, labour, delivery and infant outcomes. This information is collected prospectively during pregnancy and after childbirth and entered into the computerised systems by the attending midwife or doctor. A large amount of obstetric data are collected and are available contemporaneously. A subset of the obstetric data is extracted annually for the statewide Perinatal Data Collection (PDC). The PDC is a NSW statutory collection derived from hospital obstetric databases and homebirths, for all

livebirths or stillbirths ≥20 weeks' gestation or ≥400g birthweight.[21] The PDC includes maternal demographic characteristics, pregnancy, labour, delivery and infant outcomes such as gestational age and birthweight and is finalised on a calendar year basis.

Hospital discharge databases are accessed at both a hospital and statewide level. At the hospital level data are extracted from the Health Information Exchange which collects demographic, diagnostic and health services information for all patients admitted. Diagnoses and procedures are recorded for each hospital admission and coded by trained medical record coders according to the International Classification of Diseases 10th revision, Australian Modification (ICD-10-AM). A subset of the hospital data is extracted every three months for the statewide Admitted Patient Data Collection (APDC).[22] The APDC is a census of all inpatient hospital discharges in NSW, and includes records for both mothers and infants. The APDC has demographic, diagnostic and health services information for each admission. Up to 55 diagnoses and procedures are recorded for each hospital admission and coded according to the International Classification of Diseases. Clinical information such as pathology results or medications are not available in the APDC.

Validation studies of the obstetric and hospital databases in NSW show excellent levels of agreement with the medical record and low rates of missing data. [10-18] Reporting in both datasets has high specificity (>99%) indicating few false positive reports. Although missing data and under-reporting for some conditions does occur, only factors and outcomes accurately reported in birth or hospital data are included in analyses. [10-18] In the case of our key outcome variables, the agreement with the medical record for: preterm birth is 99.5%, labour onset (spontaneous, induction or no labour [prelabour caesarean]) 97.7%, PPROM 99.4%, induction for PPROM 98.6% and caesarean delivery 100%.[10,15,17,18]

Record linkage of study databases: To facilitate follow-up of all participants (and particularly those who birthed at a NSW hospital that was different to the recruitment hospital) the trial data will be linked to the routinely collected population health data sources (PDC birth, and mother and baby APDC hospital records). Linkage will be requested at the time of the interim analysis and again after all participants have birthed. Record linkage is conducted by an independent body, NSW Centre for Health Record Linkage (CHeReL), in accordance with NSW privacy guidelines and subject to ethics approval.[23]

Medical record reviews are undertaken to verify any cases of the primary outcome, spontaneous preterm birth. Review is performed for all pregnancies which obstetric databases record as delivering preterm, for those with no electronic record of gestational age at birth, and for a 10% random sample of those recorded as term deliveries. Those missing gestational age will include some non-viable pregnancies and also women who delivered somewhere other than their recruiting hospital and who could not be identified through record linkage. All reviews are performed by the trial coordinator, and in pregnancies where there are inconsistent records of gestational age (in completed weeks) the records are reviewed by a second researcher and a consensus reached. Where necessary, the woman's trial data will be updated to reflect findings from the medical record review. All reviews of gestational age and other data cleaning will be finalised before data lock and unblinding.

1.8 Trial endpoints

Any modifications, additions, subtractions or clarifications to the published trial protocol[20] are noted in the following sections on endpoints. Consistent with Australian national statistics, birth is used to refer to the delivery or extraction of a baby after 20^0 weeks gestation, while pregnancy loss is used to refer to pregnancies that end prior to 20 weeks.[24] Consequently, obstetric databases are limited to births ≥20 weeks. All other data sources will be utilised to minimise loss to follow-up of pregnancies that end before 20 weeks.

Some outcomes are available in multiple datasets. For those outcomes, and informed by prior validation studies, trial outcomes are extracted from data sources in the following order (i.e. if one source not available, then move to the next):

1. Obstetric databases
2. Hospital discharge databases
3. Follow up survey
4. Personal contact

It is expected that gestational age follow-up using the obstetric databases will be approximately 95%. Any preterm birth record identified in any of the obstetric or hospital databases will be subject to medical record review as specified in the preceding section.

Unless otherwise specified, the **denominator** for all analyses will be all women randomised.

1.8.1 Primary outcome: spontaneous preterm birth

The primary outcome is preterm birth (birth between 20^0-36^6 weeks gestation) following spontaneous onset of labour or following preterm prelabour rupture of membranes (PPROM) and will be analysed as a dichotomous outcome. Gestational age is based on the best clinical estimate as reported by the clinician at time of birth or miscarriage, using early ultrasound and/or menstrual dates. Obstetric databases are the primary source of information for this outcome. For women who birth outside the state of NSW the outcome will have to be obtained from the follow-up survey or personal contact.

1.8.2 Secondary outcomes

Data for the secondary outcomes are based upon the obstetric databases unless noted otherwise. If any twin pregnancies are identified after randomisation they will be included in the intention to treat analysis but neonatal outcome will be based only on the first twin.

1) *Any preterm birth <37 weeks* (birth between 20^0-36^6 weeks gestation) will be analysed as a dichotomous outcome

2) *Medically indicated preterm birth <37 weeks* (birth between 20^0-36^6 weeks gestation) will be analysed as a dichotomous outcome, and includes women with or a specific clinical indication for prelabour caesarean section (except PPROM), or a specific clinical indication for labour induction (except PPROM), and will include pregnancy terminations for congenital anomalies. Obstetric databases will be used, supplemented by hospital databases. The indications (including hypertension, growth restriction, fetal malformation, placental abnormalities, fetal death or other indications) will be reported for both trial arms. Fetal death as an indication for delivery was not specified in the trial protocol. This was an oversight.

3) *Preterm birth <32 weeks* (birth between 20^0-31^6 weeks gestation) will be analysed as a dichotomous outcome. Note that this includes any preterm birth <32 weeks, not just spontaneous preterm birth.

4) *Preterm prelabour rupture of membranes* will be analysed as a dichotomous outcome. Obstetric databases will be the primary source for this data, supplemented by the hospital databases.

5) *Spontaneous pregnancy loss <20 weeks gestation (miscarriages)* will be analysed as a dichotomous outcome. A small number of terminations of pregnancy <20 weeks may occur subsequent to randomisation and these will be reported separately. Hospital records will be the primary source for this data.

6) *Fetal growth restriction* will be analysed as a dichotomous outcome defined as birthweight < 10th centile for gestational age and sex, ie small-for-gestational-age (SGA).[25]

7) *Perinatal mortality-* includes births $\geq 20^0$ weeks gestation that resulted in either stillbirth or neonatal death (<29 days of life) and will be analysed as a dichotomous outcome. In addition to the obstetric databases, infant hospital data will also be used to identify any neonatal deaths.

8) *Admission to NICU* admission to a tertiary hospital Neonatal Intensive Care Unit will be analysed as a dichotomous outcome (yes or no). In addition to the obstetric databases, infant hospital data will also be used to identify NICU admissions.

9) *Birth weight* will be categorised as <2500 g, 2500-3999 g or ≥4000 g. This is the same way that birthweight was reported for the pilot study publication.[26]

10) *Apgar score at 5 minutes* will be analysed as a dichotomous variable defined as 5 minute Apgar<7, the same way this was reported for the pilot study publication.[26] Stillborn babies with no assigned score will be assigned a 5 minute Apgar of 0.

11) *Severe neonatal morbidity* will be determined according to a composite neonatal morbidity indicator and analysed as a dichotomous variable. The Neonatal Adverse Outcome Indicator as specified in Lain (2012) was specifically developed for assessing morbidity using routinely collected data.[27] This is the composite indicator that was specified in the trial protocol[20] and will be determined from the obstetric databases and infant hospital data (for the birth admission and subsequent transfers until discharge).

12) *Maternal length of stay (LOS) for delivery admission* will be analysed as a dichotomous variable (≥ 7 days, yes or no). This is a clarification to the 'duration of stay in hospital (maternal and infant)' in the trial protocol.[20] Maternal hospitalisation

sequences including transfers will be determined from the hospital databases as well as hospital obstetric databases.

13) *Neonatal LOS* will be analysed as a dichotomous variable (≥7 days, yes or no). This is a clarification to the 'duration of stay in hospital (maternal and infant)' in the trial protocol.[20] LOS is defined as the period of consecutive days in hospital (including transfers), commencing at birth and concluding with the date of discharge home or death. Neonatal hospitalisation sequences will be determined from the hospital databases as well as from obstetric databases

1.8.3 Secondary outcomes from the follow-up survey

A short survey is sent to participants between 28-32 weeks gestation asking about *Candida* symptoms, treatment, side effects and treatment compliance. Participation rates and data from this survey will be will be tabulated by treatment assignment and reported where relevant. This may be as text or supplementary online material in the primary publication, or as a separate publication. These outcomes include:

1) *Symptoms of candidiasis* – to assess possibility of subsequent overt infection

2) *Use of antibiotics and/or corticosteroids* – as these are risk factors for symptomatic candidiasis

3) *Treatment of candidiasis* - including study medication, doctor prescribed and over-the-counter medications to identify what proportion of the usual care group were treated

4) *Compliance with the treatment regimen* (for any women that were treated) – to identify what proportion of women completed a full course of treatment

5) *Treatment side-effects* – although these are reported in pharmaceutical product information, this will provide information for counselling should treatment prove effective.

1.8.4 Other pregnancy outcomes that occur after randomisation

1) *Pregnancy hypertension* (including gestational hypertension, preeclampsia and eclampsia), will be analysed as a dichotomous variable and obtained from the hospital obstetric databases supplemented by maternal hospital records.

2) *Gestational diabetes* will be analysed as a dichotomous variable and obtained from the hospital obstetric databases supplemented by maternal hospital records. The denominator in each arm will be pregnancies that continue beyond 28^0 weeks. Women are currently screened for gestational diabetes at approximately 28 weeks gestation so that preterm birth <28 weeks would have the appearance of being protective against GDM if the denominators are not adjusted to represent pregnancies at risk. This is a modification to the trial protocol.[20]

3) *Antepartum haemorrhage* will be analysed as a dichotomous variable based upon discharge from hospital with a relevant diagnosis, and obtained from the hospital obstetric databases supplemented by maternal hospital records.

4) *Placental abnormalities* (placenta praevia, placental abruption, morbidly adherent placenta) will be analysed as a dichotomous variable and obtained from the hospital obstetric databases supplemented by maternal hospital records.

5) *Fetal malformations* to be included will be severe and/or potentially lethal malformations: anencephaly, encephalocele, microcephaly, hydrocephalus and/or neural tube defect, cardiac defects (excluding isolated patent ductus arteriosus), oesophageal atresia, renal agenesis, congenital diaphragmatic hernia, exomphalos, gastroschisis, Trisomies 13 and 18. Note that this is a clarification to the trial protocol which only referred to congenital anomalies.[20] This outcome will be analysed as a dichotomous variable (any of the above, yes or no) and obtained from the hospital obstetric databases supplemented by maternal and infant hospital records.

6) *Onset of labour* will be analysed as spontaneous (including PPROM), induction of labour (excluding PPROM as the indication) or no labour in the case of prelabour caesarean sections (excluding PPROM as the indication) and obtained from the hospital obstetric databases.

7) *Mode of delivery* will be analysed as a dichotomous variable (vaginal birth or caesarean section) and obtained from the hospital obstetric databases.

1.8.5 Factors for subgroup analyses (for primary outcome only)

Degree of *Candida* colonisation (scant, light/moderate or heavy) based on vaginal swab at screening. Note that this is a modification to the trial protocol,[20] which specified the categories as light, moderate or heavy. Vaginal swab results received through December 2015 show that "scant" is used frequently as a description. Thus the protocol definition of this subgroup has been modified to better reflect current clinical practice.

Candida albicans (with or without non-albicans species) compared with *non-albicans* only species based on vaginal swab at screening will be analysed as a dichotomous variable obtained from the CiPS database. Note that this is a clarification to the trial protocol, which did not specify how samples reported to have both albicans and non-albicans species would be categorised.

Gestational age at time of randomisation will be analysed as a dichotomous baseline factor (<14 weeks [1st trimester] compared with ≥14 weeks [2nd trimester]). Note that this is a clarification to the trial protocol, which did not specify how gestational age would be categorised.

Medically indicated preterm births by indication note that this group is mutually exclusive from the primary outcome so cannot be reported as a subgroup analysis although this was proposed in the original protocol.

2. STATISTICAL ANALYSIS

2.1 Study size

The study protocol specified a sample size of 3208 women with *Candida* colonisation required to detect a 40% reduction in spontaneous preterm births among women with asymptomatic candidiasis from 5% in the control group to 3% for those treated with clotrimazole (significance 0.05, power 0.8). It was anticipated that 16,040 women would need to be screened based on a *Candida* carriage rate of 20%.

2.2 Participant flow diagram

A CONSORT (Consolidated Standards of Reporting Trials) type diagram will be used to show the flow of participants into the final analysis.[28] This is expected to be Figure 1 (see Section 3, Figure 1).

We will also report the preterm birth rate among women who were screened and their swab indicated that there was no colonisation with *Candida*.

2.3 Analysis Principles

All analysis will be by intention to treat. Analyses will follow the CONSORT guidelines.[28] Any known protocol violations will be reported in the text, but participants will not be excluded from the intention to treat analysis for protocol violations. This includes women who are identified post-randomisation as having a twin pregnancy and women whose gestational age at screening was 20 weeks. The number of participants lost to follow-up will be reported.

Preliminary analysis of baseline variables will be conducted using blinded data. Unblinding of treatment assignment will not occur until the statistical analysis plan has been finalised and the dataset is locked.

An alpha value of 0.0497 will be used in light of the interim analysis, to preserve the overall p-value of the study at 0.05 using the O'Brien-Fleming method.[29]

2.3.1 Distribution of baseline variables

Maternal demographics and pregnancy characteristics at randomisation will be reported in Table 1 (see page 19).

2.3.2 Missing baseline variables

No imputation will be conducted for missing baseline factors in the main analyses. If information on maternal age or previous pregnancy and previous preterm is missing it may be obtained from linked prior birth records. Imputation will be used for the adjusted analysis of the primary outcome (see Section 2.3.5). Imputation for missing values will be performed based upon women's characteristics by age, parity and recruiting hospital.

2.3.3 Missing primary outcome

There will be no imputation for missing primary outcome. Medical record review will be conducted where gestational age at birth is missing.

2.3.4 Unadjusted analysis of primary outcome

The proportions of spontaneous preterm birth in the treatment and control arms will be compared using chi-square tests. Relative risks and confidence intervals will be reported using the usual care group as the comparison group (Table 2). An alpha value of 0.0497 will be used in light of the interim analysis, to preserve the overall p-value of the study at 0.05 using the O-Brien-Fleming method.[29]

Over the course of the study, it is possible that the same woman may be enrolled more than once (subsequent pregnancies). If the number of women randomised multiple times exceeds 5% of the total randomised, then multilevel models will be used to account for clustering by woman.

2.3.5 Adjusted analysis of primary outcome

A multilevel regression model will be used to perform an adjusted analysis of the effect of treatment upon the primary outcome. The recruiting hospital will be included in the model as the second level hierarchical variable. Any of the other baseline factors shown in Table 1 for which there is a significantly different distribution (P<0.05) between treatment arms will also be included in the model as a covariate. If included, the covariate factors would be categorised as follows: maternal age at randomisation (<25, 25-34,35+); gestation at randomisation (categorical, ≤12, 13-14, 15-16, 17-18, ≥19 weeks), previous preterm birth (nullipara, multipara with no previous preterm birth, multipara with previous preterm birth), highest level of education (secondary school/post-secondary school), colonisation (scant, light/moderate, heavy), *Candidia* species (albicans, non-albicans only), and prior treatment for vaginal thrush (ever or this pregnancy - yes/no).

2.3.6 Missing secondary outcomes

There will be no imputation for missing secondary outcomes.

2.3.7 Analysis of secondary outcomes

Dichotomous outcomes will be reported (Table 2) as number of events and percent per arm, and compared using relative risks and confidence intervals, using the usual care group as the comparison group. No adjustment to the level of significance will be made for multiple comparisons. Secondary neonatal outcomes will use the number of liveborn infants in each arm as the denominators for analyses.

2.3.8 Other pregnancy and delivery outcomes

Additional dichotomous pregnancy outcomes will be reported in Table 3 as number of events and percent per arm, and compared using relative risks and confidence intervals, using the usual care group as the comparison group.

2.3.9 A priori subgroup analyses

Pre-specified subgroup analyses are presented in Table 4. Sub-group analyses will examine the relationship between spontaneous preterm birth and clotrimazole treatment by degree of *Candida* colonisation (Scant, Light/Moderate, Heavy); *Candida albicans* (with or without other candida species) versus *non-albicans only* species, and gestational age at randomisation (<14 weeks versus ≥14 weeks). These pre-specified subgroup analyses will be undertaken for the primary outcome (spontaneous preterm birth) only (clarification of the published protocol which simply states for 'preterm birth').[20] Chi-square tests for interaction will be used to test for differences, with the trend version used for degree of colonisation.

2.3.10 Post-hoc hypotheses generating subgroup analyses

Any subgroup analyses not specified above will be clearly identified as such in any reporting of the trial.

2.3.11 Sensitivity analyses

A sensitivity analysis excluding any severe fetal malformations (as defined in the outcomes section) or any terminations will be performed.

If more than 0.5% of the randomised pregnancies are multiple pregnancies, a sensitivity analysis which excludes multiple pregnancies will be performed.

2.4 Interim Analysis

As specified in the published study protocol,[20] an interim analysis of the primary outcome (spontaneous preterm birth) and perinatal mortality will be conducted when birth outcome data become potentially available for the first 50% of women recruited. At this point, in addition to the regular monitoring of recruitment, losses to follow up, data completeness and quality, treatment side-effects and harms, and the impact of external evidence on the study, the Data Monitoring Committee (DMC) will make a de-identified (Group A versus Group B) assessment of the treatment effect on the primary outcome and review the sample size assumptions. However, treatment allocation will be revealed to the DMC on request.

The possible recommendations by the DMC include:
- No action needed, trial continues as planned
- Early stopping due, for example, to clear benefit or harm of a treatment, futility, or external evidence
- Extending recruitment or extending follow-up
- Sanctioning and/or proposing protocol changes.

The Steering Committee will make the final decision of whether to continue or suspend the study.

The interim analysis will follow the analysis principles and definitions outlined in this document. The O'Brien Fleming method[29] will be used to ensure the overall P-value for the study remains at alpha=0.05, with the interim analysis tested at alpha=0.0031.

3. DUMMY TABLES AND FIGURES

Table 1 Maternal and pregnancy factors at or before time of randomisation

	Treatment N= n (%)	Control N= n (%)
Gestation at randomisation		
<14^0 weeks		
14^0 to 15^6 weeks		
16^0 to 17^6 weeks		
≥18^0		
Dating ultrasound (<20 weeks)		
Highest level of education		
Secondary school		
University		
Other post-secondary		
Maternal age (years) (mean, std.dev.)		
Parity		
Nulliparous		
Multiparous		
Previous preterm birth		
Yes		
No		
Type of previous preterm birth*		
Spontaneous labour/PPROM†		
Indicated		
Gestation of previous preterm birth		
<32^0 weeks		
32^0-36^6 weeks		
Prior treatment for vaginal thrush (ever)		
Yes		
No		
Vaginal thrush treatment this pregnancy		
Yes		
No		
Degree of colonisation		
Scant		
Light		
Moderate		
Heavy		
Candida species		
Candida albicans (any)		
Non-*albicans* only		

*   Multiple responses (for >1 previous birth) allowed

†   Preterm Prelabour Rupture of Membranes

Percentages may not add to 100% because of missing data

Table 2 Pregnancy, neonatal and maternal outcomes by treatment assignment

Primary outcome	Treatment N= n (%)	Usual Care N= n (%)	Relative risk RR (95% CI)
Spontaneous preterm birth (<37 weeks)*			
Secondary outcomes			
Any preterm birth (<37 weeks)			
Medically indicated preterm birth, for Hypertension Intrauterine growth restriction Fetal malformation Placental abnormalities Fetal death Other			
Preterm birth <32 weeks			
Preterm prelabour rupture of membranes			
Spontaneous pregnancy loss <20 weeks			
SGA (<10th centile for gestational age)			
Birthweight (grams) <2500 2500-3999 ≥4000 g.			
5 minute Apgar <7			
Perinatal mortality			
Maternal LOS ≥7 days			
Secondary neonatal outcomes among liveborn infants	n=	n=	
Admission to NICU			
Neonatal adverse outcome indicator†			
Neonatal LOS 7 ≥ days			

* includes any birth after preterm prelabour rupture of membranes (PPROM)
† including but not limited to respiratory distress, assisted ventilation, intraventricular haemorrhage, necrotising enterocolitis, retinopathy and pneumonia[27]
LOS=length of stay; NICU=neonatal intensive care unit admission

Table 3 Other pregnancy and birth outcomes, by treatment assignment

	Treatment n (%)	Usual Care n (%)	Relative risk RR (95% CI)
Pregnancy hypertension			
Gestational diabetes*			
Antepartum haemorrhage			
Placental abnormalities			
Fetal malformations			

Onset of labour
  Spontaneous†
  Induction
  No labour			

Mode of delivery
  Vaginal birth
  Caesarean section			

* Among women who reach at least 28 weeks of gestation
† includes any birth after preterm prelabour rupture of membranes (PPROM)

Table 4: Pre-specified subgroup analyses for preterm birth (<37 weeks)

	Treatment Preterm birth n/N (%)	Usual care Preterm birth n/N (%)	Preterm birth RR (95%CI)	P for interaction
Degree of Colonisation				
Scant				
Light/moderate				
Heavy				
Candida species				
Albicans (any)				
non-*albicans* only				
Gestational Age at randomisation				
<14 weeks				
≥14 weeks				

Figure 1 CONSORT Flow diagram for the Candida in Pregnancy Study

Enrolment

Assessed for eligibility
(n=)

Declined (n=)

Vaginal swab collected
(screened)
(n=)

Asymptomatic but positive
for *Candida species*
Randomised
(n=)

Negative for *Candida species*
(n=)

Randomisation

Allocated to treatment (n=)
Dispensed treatment (n=)

Allocated to usual care (n=)

Follow Up

Lost to follow up (n=)

Lost to follow up (n=)

Analysis

Analysed (n=)
Excluded (n=)
Reasons:
(x women revoked consent)

Analysed (n=)
Excluded (n=)
Reasons:
(x women revoked consent)

4. ADDITIONAL ANALYSES

4.1 Characteristics of women with and without asymptomatic Candidiasis

Amongst all women who were screened for *Candida* sp. and who gave consent to record linkage, a further set of analysis may be conducted. This would compare maternal and pregnancy characteristics and outcomes among women with asymptomatic candidiasis (randomised participants) and those without candidiasis (women not randomised into the trial). This study will be undertaken separately.

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Appendix 1

CiPS Baseline Data Collection Form

PaLMS
Pacific Laboratory Medicine Services

CiPS Candida in Pregnancy Study S450
CLINICAL TRIAL PATHOLOGY REQUEST FORM
Use ONLY to order pathology for this Clinical Trial

Patient Study ID: CIPS01000

CiPS Clinical Trial Coordinator
Name: Kristen Rickard
         Perinatal Research
         Building 52, Reserve Rd
         Royal North Shore
         St Leonards NSW 2065

Phone: (02) 9926 6142
Email: krrickar@nsccahs.health.nsw.gov.au

COLLECTION DETAILS

COLLECTION DATE: …..… / …..… / …..…
                      dd   mm   yy

COLLECTION TIME: …..… : …..…
                      hh   mm

Recruiting Doctor/Midwife:……………………………………..

DATA ENTRY CODES

HCF:S450   **WARD: S450**   **DOCTOR: S450**   **BILLING CATEGORY: S450**

TEST REQUEST *Delete tests if not required*	SPECIMEN GUIDE	LAB USE				
		Test Entry Code	Testing Laboratory/ Specimen Handling Instructions	Specimen Received	Staff Check	Lab no
Detection and identification of *Candida*	1 x Vaginal Swab in Amies Transport Media	SW Vag Genit (chrom only)	Microbiology Laboratory - semiquantitative fungal culture and limited speciation (CHROMagar plate)			

PRINCIPAL INVESTIGATOR: Prof Jonathan Morris   Phone: 02 9926 7013

PLEASE NOTE: *Routine collections on the same patient MUST be sent with the standard PaLMS request form.*
For further information, please contact the PaLMS Clinical Trial Co-ordinator: (02) 8425 3126
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For Researcher Use Only

Please use Pt Addressograph if available

Patient Details   Hospital ……………………….....................

MRN…………………………………………...DOB: ….. / ….. / …...

Surname………………………………………………………....

Given Name: …...…………………………………………….

Address ………………………………………………………..

…………………………………… Postcode…………………...

Home ph……...……….……Mob/wk……………………………..

Email ………………………………………………………....

Alternate Contact Details…………………………………….

…………………………………………………………………....

Preferred method of contact:
                    Home □   Mob/wk □   Email □

Highest level of completed education:
Statistical Analysis Plan July 2016 University □

1) LMP   ..…… / ..... /….. or unknown □
            dd   mm   yy

2) Gestation at trial entry: _____ wks /___days

3) EDB   ...…… / ……. / …...
            dd   mm   yy

4) Early U/S ...…… / ……. /……. & Gest ___wks/__ days
            dd   mm   yy

5) Have you been pregnant before: Yes / No
    If yes, how long since your last pregnancy
    (ended)………….. mths/yrs

6) Previous preterm birth (20 -<37wks): Yes / No
    .. .If yes, gestation at delivery: ……….. (wks)
       & was the preterm birth (tick all that apply)

Spont Labour □   PPROM □ or Medically indicated □

7) Have you received treatment for (vaginal?) thrush?
       i) At any time in your life   Yes / No
       ii) During this pregnancy Yes / No